Supplementary data

RNA isolation and quantitative PCR

Gene expression analysis was conducted on GABAergic forebrain neurons. RNA was isolated with a NucleoSpin RNA kit (Macherey-Nagel Düren, Germany). The concentration and purity of RNA were quantified with a NanoDrop 1000 (Thermo Fisher Scientific). The 200-800 ng reaction of RNA was converted to cDNA using a High-Capacity cDNA ReverseTranscription Kit (Themo Fisher Scientific). The expression levels of all genes of interest (Supplementary Table S1) were analysed with TaqMan assays using an ABI Prism 7300 real-time PCR system (Thermo Fisher Scientific). Each 15 µl reaction contained 15 ng cDNA, 0.75 µl 20 ×TaqMan Gene Expression Assay and 7 µl 2 × TaqMan Gene Expression Master Mix. The data were analyzed using the delta-delta Ct method using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control [46]. RNA samples were prepared from two separate experiments and in each experiment, RNA was collected at DIV 0, 12, 19, 37, and 94. All the samples were run in triplicate and data are presented as average +/- SEM.

Immunocytochemistry

Immunocytochemistry was performed as previously described [47]. Briefly, samples were blocked with 10% normal donkey serum, 0.1% Triton X-100 and 1% bovine serum albumin in PBS, at room temperature for 45 min. Next, the samples were incubated overnight at + 4 ◦C with primary antibodies listed in Supplementary Table S2. Thereafter, samples were incubated at RT for an hour with Alexa Fluor-labelled secondary antibodies, (Supplementary Table S3). For fluorescence imaging, an Olympus IX51 inverted fluorescence microscope was used (Olympus). Confocal imaging was performed using an LSM 800 laser scanning confocal microscope (Zeiss). For cortical networks confocal image analysis on glutamatergic (VGLUT1) and GABA-specific (GAD67) markers, and pre-and post-synaptic markers, Synaptophysin and PSD95, were deconvolved using the Huygens essential software (Scientific Volume Imaging, Hilversum, Netherlands) prior to analysis in IMARIS software 10.1.0 (Oxford Instruments). A minimum of 10 field-of-view images were taken per coverslip of each cell line. Briefly, with Imaris software, the number of puncta was quantified using the 'spots' function. The size of spots detection was with an XY diameter ranging from 0.6-0.8µm and a point spread function Z-diameter of 1.5µm. Neurites were reconstructed into 'surfaces' and smoothed using a Gaussian filler (minimum size: 0.3). Spots were filtered with a threshold distance of 0.5 µm close to the 'surface' starting from the center point of the surface. The filament tracing function was used to determine neurite length.

MEA data analysis

Spike detection for single channel analysis was performed with a previously described custom MATLAB (MathWorks) script [29,33]. Bursts, presenting the local population activity, were identified locally at single channel level from the spike timestamps with the logISI algorithm [34]. Spike and burst features and other

parameters such as the number of active electrodes (electrodes with > 10 spikes/min) were computed with R-package, meaRtools per well [35]. Burst features were presented only for burst-detecting electrodes. Briefly, the algorithm separates network firing regimes from irregular firing using interspike interval (ISI) distribution between N consecutive spikes. Initially, the time points of all detected spikes from all active electrodes in a well are combined into a logarithmic ISIN histogram. In cases of synchronous network activity, this histogram tends to exhibit two peaks representing two different firing regimes, i.e., spikes belonging to network bursting and spikes outside of network firing, respectively. Thus, the ISIN threshold for detecting network bursts is selected at the minimum point between these peaks.

For network burst detection: Adaptive merging is performed based on durations of neighbouring network bursts. The duration of the longest of those is multiplied by a merging fraction and if the interburst interval (IBI) is less than the obtained value, the network events are merged. Moreover, each network burst instance requires contribution from at least 3 channels in a well. The sufficiency is defined as a fraction of mean channel contribution within the detected network burst instance. In this study N was set to 20, the merging fraction to 0.5 and the fraction of mean channel contribution to 0.3.

Principal component analysis (PCA) was performed according to [48] using MATLAB software with custom scripts in order to differentiate control and DS networks based on 17 different MEA Features: Mean firing rate (MFR), total spikes, burst per min, number of bursts (nbursts), burst duration, interburst intervals (IBIs), mean spike frequency in bursts, mean amplitude, number of network bursts (NBs), mean duration of NBs, mean spike frequency in NBs, percentage of spikes in NBs, mean spikes in NBs, mean channels in NBs, mean dominating channels in NBs, mean IBIs in NBs. (Compiled from both single channel level and Network level features. The selected timepoints used for GABAergic networks were at DIV 68 and 82 from all cultured networks (controls 1-3 and DS patients). DIV67 and 83 were timepoints used in cortical networks from controls 2, 4 and DS patients. PCA was performed using MATLAB software and plotted against three explanatory principal components (PCA 1- 3). The top 5 features of the different principal components were identified according to highest absolute loading values for each feature (parameter) in that principal component (Supplementary Table S4).

The initial three principal components (PC 1-3) were visually represented through a 3D scatter plot using the Matlab scatter3 function where each data point represents separate MEA wells. Employing the Matlab *alphaShape* function, we constructed boundaries around the data points; thus, visualizing a surface, encapsulates the group of data points and provides automatic identification of clusters associated to each cell line. An alpha radius of 2.6 is set for this purpose. Generated boundaries facilitate the calculation of overlap between the clusters of different cell lines, which quantifies the spatial concurrence between the PCs of various groups. The percentage overlap (%*Overlap*) is calculated by measuring the containment of points from one group within the clusters of other groups. An example expressed as follows:

$$\%Overlap_{Control\ 1,Control\ 2} = \frac{number\ of\ points\ from\ Control\ 1\ inside\ cluster\ of\ Control\ 2}{total\ number\ of\ points\ in\ Control\ 1}\ x\ 100$$

GABA ELISA

GABA secretion from GABAergic enriched on DIV 96 and heterogenous cortical cultures on DIV 133 was analyzed using a commercial human GABA ELISA kit (Abcam, AB287792) following the manufacturer's instructions and analyzed using a plate reader Viktor Nivo at 450 nm (PerkinElmer, Supplementary Fig. S3). Media supernatant was collected from the MEA plates on ice with centrifugation at 10000 × g for 10 min at 4 °C to remove insoluble debris and dead cells and stored at -80 °C until analyses.

Supplementary Table S1: List of primers used.

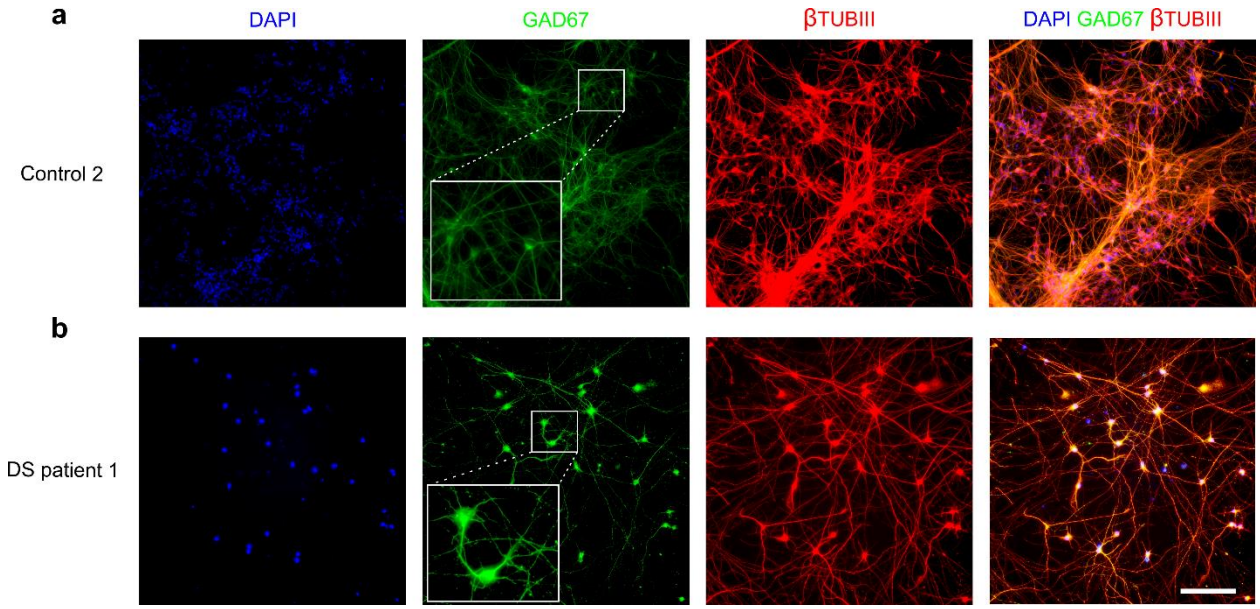
Gene name	Gene ID	Source
NKX2.1	Hs00968940_m1	Thermo fischer Scientiifc
LHX6	Hs01030943_m1	Thermo fischer Scientiifc
DLX2	Hs00269993_m1	Thermo fischer Scientiifc
SCN1A	Hs00374696_m1	Thermo fischer Scientiifc
GAD67 (GAD1)	Hs01065893_m1	Thermo fischer Scientiifc
SIX6	Hs00201310_m1	Thermo fisher Scientific
NKCC1	Hs00169032_m1	Thermo fisher Scientific
KCC2	Hs00221168_m1	Thermo fisher Scientific

Supplementary Table S2: List of antibodies used.

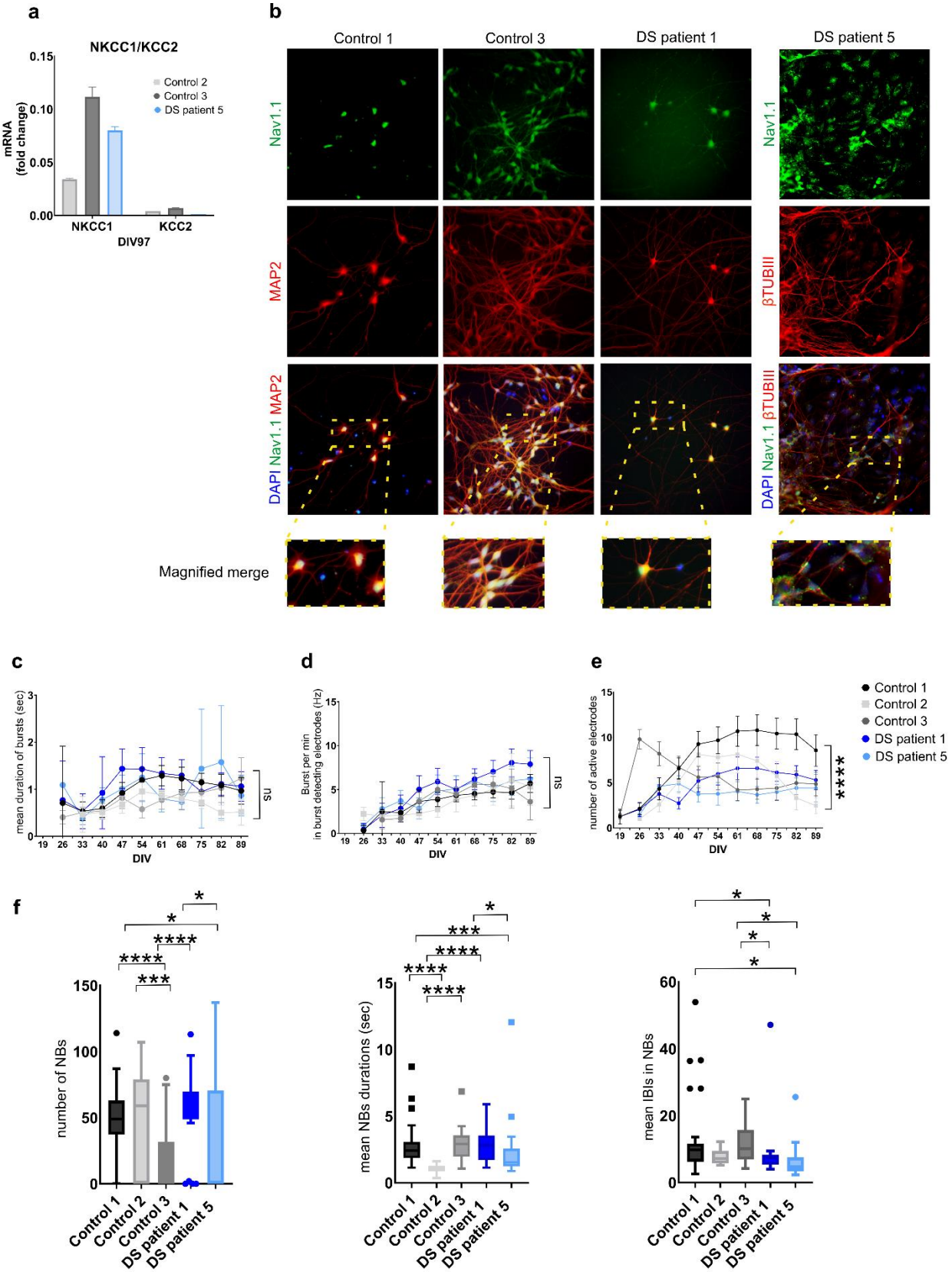
Antibody	Dilution	Species	Supplier
βTUBIII	1: 200	chicken	Abcam: ab41489
FOXG1	1:500	rabbit	Abcam: ab18259
GAD67	1:100	mouse	Millipore: MAB5406
MAP2	1:400	chicken	Novus Biologicals: NB300-213
MAP2	1:400	rabbit	Millipore: AB5622
OCT4	1:200	goat	R&D systems Inc: AF1759
Pax6	1: 1000	rabbit	BioLegend: 901301
Sox2	1:200	mouse	R&D systems Inc: MAB2018
Synaptophysin	1 :500	rabbit	Abcam: ab32127
Nav1.1	1:500	rabbit,	Abcam: ab24820
vGlut1	1:2000	rabbit	Synaptic Systems: 135303
vGlut2	1:1000	guinea pig	Milipore AB2251

Supplementary Table S3: List of Alexa Fluor-labelled secondary antibodies used.

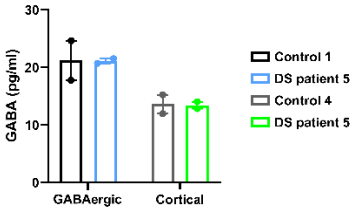
Species raised	Dilution	Supplier. All from Thermo Fischer Scientific
donkey anti-rabbit 488	1:400	A21206
donkey anti-mouse 488	1:400	A21202
donkey anti-rabbit 568	1:400	A10042
donkey anti-mouse 568	1:400	A10037
goat anti-chicken 568	1:400	A11041
donkey anti-rabbit 647	1:200	A31573
goat anti-chicken 647	1:200	A21449



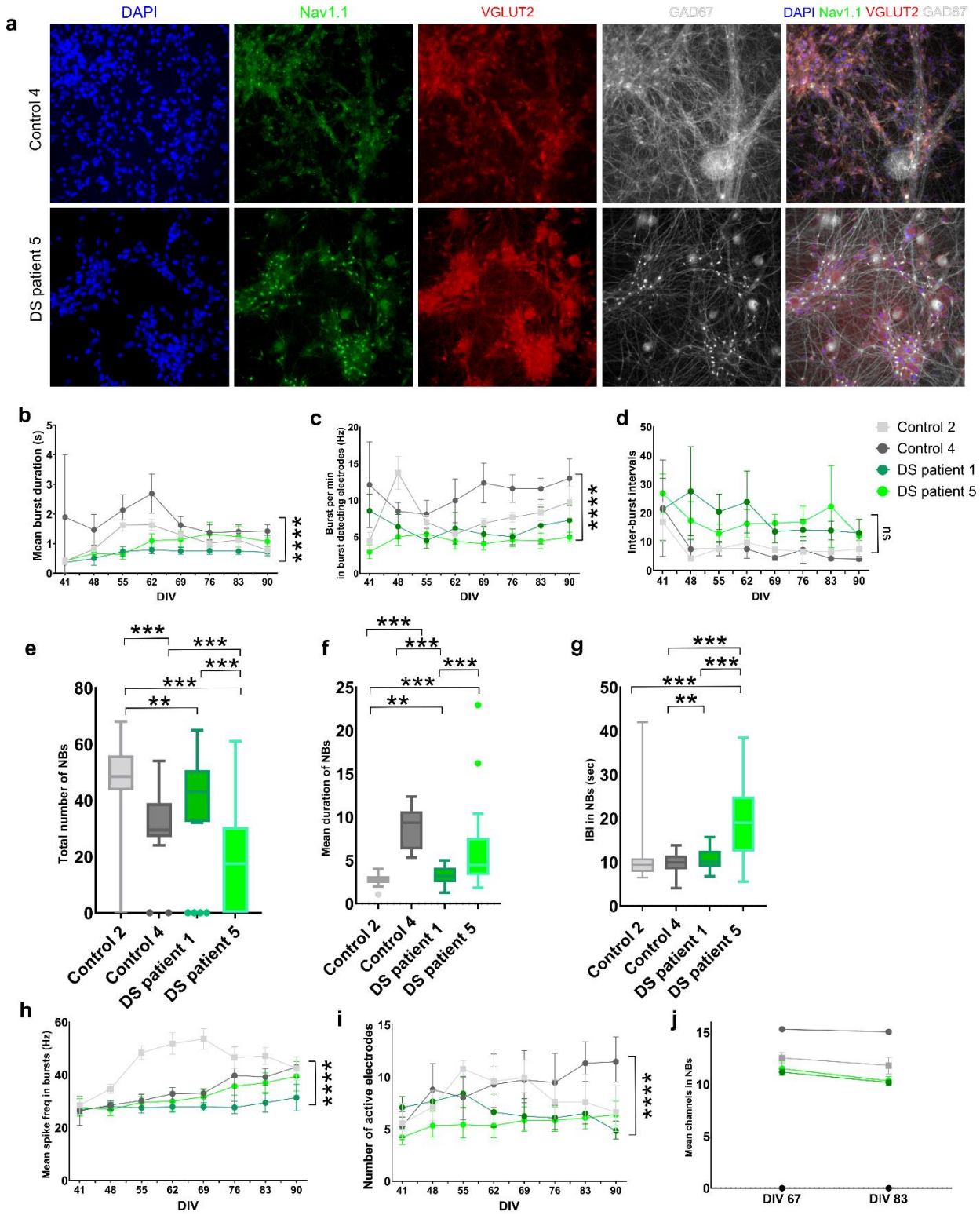
Supplementary Fig S1. **Neuronal maturation and GABAergic identity.** Immunocytochemistry showing maturation and GABAergic neuron identity by DIV 37 for (**a**) Control 1 and (**b**) DS patient 1. Nuclei were counterstained with DAPI (blue), axons were counterstained with βTUBIII and GABAergic neurons with GAD67. The scale bar is 200 µm.


a
NKCC1/KCC2
mRNA (fold change)
0.15
0.10
0.05
0.00
Control 2
Control 3
DS patient 5
NKCC1
KCC2
DIV97
b
Control 1
Control 3
DS patient 1
DS patient 5
Nav1.1
MAP2
βTUBIII
DAPI Nav1.1 MAP2
DAPI Nav1.1 βTUBIII
Magnified merge
c
mean duration of bursts (sec)
ns
DIV
d
Burst per min in burst detecting electrodes (Hz)
ns
DIV
e
number of active electrodes
****
DIV
Control 1
Control 2
Control 3
DS patient 1
DS patient 5
f
number of NBs
mean NBs durations (sec)
mean IBIs in NBs
Control 1
Control 2
Control 3
DS patient 1
DS patient 5

Supplementary Fig. S2. **Characterization of GABA differentiation and functional development.** (**a**) Graph showing gene expression of co-chloride transporters, NKCC1 and KCC2 in GABAergic forebrain, at DIV97 of differentiation. (**b**) Representative immunocytochemical staining showing expression of Nav1.1 in cultures at DIV 37 of the GABA differentiation protocol. (**c**) Graph showing mean duration of bursts during development, (**d**) burst per min in burst detecting electrodes and (**e**) number of active electrodes at the single channel level. (**f**) Graph showing number of NBs counts, mean NBs duration and mean IBIs in NBs at DIV68. For all MEA data shown, *n*=number of wells/independent differentiations: Control 1 *n*=44/3, Control 2 *n*=16/1, Control 3 *n*=28/2, DS patient 1 *n*=30/2, DS patient 5 *n*=52/3. Data in graphs c-e is shown as mean±95% confidence interval (CI) and statistical analyses were conducted with mixed-effects model with Tukey's multiple comparisons over time comparisons of networks (cell lines). The detailed statistical differences between the cell lines are shown in Supplementary Table S8. In graph f, data is presented as mean with SEM or as Tukey box plots. Statistical analysis was performed using one way ANOVA with Kruskal-Wallis tests for multiple comparison of cell lines. Mann-Whitney U test was used to determine differences between two cell lines (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p \leq 0.0001$).



Supplementary Fig. S3. Graph showing the secretion of GABA from GABAergic enriched cultures on DIV96 (Control 1 and DS patient 5) and from heterogeneous cortical cultures on DIV133 (Control 4 and DS patient 5). N=2 for all the groups and data is shown as mean±SEM.


a
DAPI
Nav1.1
VGLUT2
GAD67
DAPI Nav1.1 VGLUT2 GAD67
Control 4
DS patient 5
b
Mean burst duration (s)
DIV
****
c
Burst per min in burst detecting electrodes (Hz)
DIV
****
d
Inter-burst intervals
DIV
ns
Control 2
Control 4
DS patient 1
DS patient 5
e
Total number of NBs
***
**
Control 2
Control 4
DS patient 1
DS patient 5
f
Mean duration of NBs
***
**
Control 2
Control 4
DS patient 1
DS patient 5
g
IBI in NBs (sec)
***
**
Control 2
Control 4
DS patient 1
DS patient 5
h
Mean spike freq in bursts (Hz)
DIV
****
i
Number of active electrodes
DIV
****
j
Mean channels in NBs
DIV 67
DIV 83

Supplementary Fig. S4. **Functional characterization of heterogenous cortical cultures.** (**a**) Immunocytochemical staining showing protein expression of Nav1.1 in control and diseased networks. (**b**) Graph showing mean burst duration, (**c**) burst per minute, (**d**) IBI during development. (**e**) Quantitative NBs data showing number of NB counts, (**f**) mean NBs duration and (**g**) IBIs between NBs at DIV 67. (**h**) Graph showing mean spike frequency in bursts at the single channel level. (**i**) Number of active electrodes and (**j**) mean channels (electrodes) participating in NBs. For all MEA data shown, *n*=number of wells/separate differentiation: Control 1 *n*=40/2, Control 2 *n*=28/2, Control 4 *n*=40/3, DS patient 1 *n*=60/4, DS patient 5 *n*=52/4. Data in graphs b-d and h-i is shown as mean±95% confidence interval (CI) and statistical analyses were conducted with mixed-effects model with Tukey's multiple comparisons over time comparisons of networks (cell lines). The detailed statistical differences between the cell lines are shown in Supplementary Table S10 and S11. In graphs e-g, data is presented as mean with SEM and median Tukey box plots. Statistical analysis was performed using one way ANOVA with Kruskal-Wallis tests for multiple comparisons. Mann-Whitney U test was used to determine differences between two cell lines (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p \leq 0.0001$).

Supplementary Table S4: Prominent MEA features contributing to the different principal components.

GABAergic enriched cultures		
PC1	PC2	PC3
Total spikes	Total number of NB	burst duration
MFR	Burst rate	Mean amplitude
Mean spike frequency in NB	nbursts	Percentage Of spikes in bursts
nbursts	Percentage Of spikes in bursts	Mean IBI
Mean spikes in NB	Percentage Of spikes in NB	Burst rate
Heterogeneous cortical cultures		
PC1	PC2	PC3
Total spikes	Total number of NB	Mean dominating channels in NB
MFR	Burst rate	Percentage Of spikes in NB
nbursts	Mean amplitude	Mean channels in NB
Mean spike frequency in NB	nbursts	nbursts
Mean spikes in NB	MFR	Total number of NB

nbursts= number of bursts, NB=network bursts, IBI= inter-burst-intervals, MFR=mean firing rate

Supplementary Table S5: %Overlap of enriched GABAergic cultures in PCs.

	Control 1	***Control 2***	***Control 3***	***DS patient 1***	***DS patient 5***
Control 1	98.438	3.125	12.500	18.750	1.563
Control 2	29.411	100	11.765	47.059	29.412
Control 3	14.286	19.048	100	33.333	14.286
DS patient 1	19.048	9.524	28.571	100	14.286
DS patient 5	3.030	12.121	3.030	36.363	96.970

Supplementary Table S6: %*Overlap* of heterogeneous cortical cultures in PCs.

	Control 2	***Control 4***	***DS patient 1***	***DS patient 5***
Control 2	100	28.846	3.846	21.153
Control 4	5	100	0	0
DS patient 1	7.018	3.509	100	26.316
DS patient 5	3.636	1.818	23.636	100

Supplementary Table S7: Statistics of MFR for active electrodes, nbursts and percentages of spikes in bursts in GABAergic enriched cultures.

Tukey's multiple comparisons test		Figure 2A		Figure 2B		Figure 2C	
		MFR for active electrodes		**nburst**		**Percentages of spikes in burts**	
		Sig	Adj. p	Sig	Adj. p	Sig	Adj. p
DIV19	**-**	-	-	-	-	-	-
DIV26	DS patient 1 vs Control 1	ns	0.59	ns	0.9203	ns	>0.9999
	DS patient 1 vs Control 2	ns	0.3419	ns	0.487	ns	0.621
	DS patient 1 vs Control 3	ns	>0.9999	***	0.0006	ns	0.5318
	DS patient 5 vs Control 1	ns	0.7013	ns	0.9993	ns	0.9891
	DS patient 5 vs Control 2	ns	0.5728	ns	0.8496	ns	0.8496
	DS patient 5 vs Control 3	ns	0.988	***	0.0005	ns	0.999
	DS patient 1 vs DS patient 5	ns	0.9905	ns	0.9896	ns	0.9927
	Control 1 vs Control 2	ns	0.971	ns	0.5629	ns	0.6299
	Control 1 vs Control 3	ns	0.1192	***	0.0005	ns	0.4143

	Control 2 vs Control 3	*	0.026	***	0.0004	****	<0.0001
DIV33	DS patient 1 vs Control 1	ns	0.9026	ns	0.9847	ns	0.6995
	DS patient 1 vs Control 2	ns	0.5521	ns	0.9797	ns	0.3786
	DS patient 1 vs Control 3	ns	>0.9999	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 1	ns	0.876	ns	0.7769	ns	0.377
	DS patient 5 vs Control 2	ns	0.0945	ns	0.6632	ns	0.3948
	DS patient 5 vs Control 3	ns	0.9997	****	<0.0001	****	<0.0001
	DS patient 1 vs DS patient 5	ns	>0.9999	ns	0.9837	ns	0.9998
	Control 1 vs Control 2	ns	0.4243	ns	>0.9999	ns	0.1734
	Control 1 vs Control 3	ns	0.8914	****	<0.0001	****	<0.0001
	Control 2 vs Control 3	*	0.0194	****	<0.0001	ns	0.8063
DIV40	DS patient 1 vs Control 1	ns	0.0543	ns	0.9799	ns	0.9966
	DS patient 1 vs Control 2	*	0.0383	ns	0.7191	***	0.0008
	DS patient 1 vs Control 3	*	0.0119	ns	0.4317	ns	0.073
	DS patient 5 vs Control 1	ns	0.4004	ns	0.2868	*	0.0257
	DS patient 5 vs Control 2	ns	0.3167	ns	0.7792	ns	0.213
	DS patient 5 vs Control 3	ns	0.1229	ns	0.9916	ns	0.9761
	DS patient 1 vs DS patient 5	ns	0.8933	ns	0.2388	*	0.0317
	Control 1 vs Control 2	ns	0.9993	ns	0.8898	***	0.0009
	Control 1 vs Control 3	ns	0.6758	ns	0.5614	ns	0.0598
	Control 2 vs Control 3	ns	0.7337	ns	0.9612	ns	0.0739
DIV47	DS patient 1 vs Control 1	*	0.0233	ns	0.8787	ns	0.1285
	DS patient 1 vs Control 2	ns	0.4155	ns	0.9559	ns	0.7576
	DS patient 1 vs Control 3	***	0.0003	ns	0.0727	**	0.0013
	DS patient 5 vs Control 1	ns	0.6102	***	0.0002	ns	0.5303
	DS patient 5 vs Control 2	ns	0.1257	****	<0.0001	ns	0.0717
	DS patient 5 vs Control 3	ns	0.7397	ns	0.8674	ns	0.9997
	DS patient 1 vs DS patient 5	**	0.0018	*	0.0436	**	0.003
	Control 1 vs Control 2	ns	0.6455	ns	0.9844	ns	0.7421
	Control 1 vs Control 3	ns	0.1349	**	0.0013	ns	0.3851
	Control 2 vs Control 3	*	0.0257	***	0.0002	*	0.0392
DIV54	DS patient 1 vs Control 1	ns	0.4295	ns	0.9953	ns	0.9039
	DS patient 1 vs Control 2	ns	0.5608	ns	0.9961	ns	0.9362
	DS patient 1 vs Control 3	**	0.0064	ns	0.0809	****	<0.0001
	DS patient 5 vs Control 1	ns	0.2875	****	<0.0001	*	0.0351
	DS patient 5 vs Control 2	*	0.0186	***	0.0009	***	0.0006
	DS patient 5 vs Control 3	**	0.0073	ns	0.9748	ns	0.5672
	DS patient 1 vs DS patient 5	ns	0.0738	*	0.0433	**	0.0025
	Control 1 vs Control 2	ns	0.9756	ns	0.8399	ns	0.5262
	Control 1 vs Control 3	***	0.0003	***	0.0005	****	<0.0001
	Control 2 vs Control 3	****	<0.0001	**	0.0039	****	<0.0001
DIV61	DS patient 1 vs Control 1	ns	0.9981	ns	0.6721	ns	0.633
	DS patient 1 vs Control 2	ns	0.9954	ns	0.9997	ns	0.9317

	DS patient 1 vs Control 3	**	0.0061	*	0.0205	***	0.0001
	DS patient 5 vs Control 1	*	0.0131	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 2	*	0.0228	*	0.0222	**	0.0034
	DS patient 5 vs Control 3	*	0.0373	ns	0.7113	ns	0.9893
	DS patient 1 vs DS patient 5	ns	0.0816	ns	0.107	**	0.0015
	Control 1 vs Control 2	ns	>0.9999	ns	0.3463	ns	>0.9999
	Control 1 vs Control 3	****	<0.0001	****	<0.0001	****	<0.0001
	Control 2 vs Control 3	***	0.0005	**	0.0015	***	0.0007
DIV68	DS patient 1 vs Control 1	ns	0.9925	ns	0.4061	ns	0.9991
	DS patient 1 vs Control 2	ns	0.9992	ns	0.9956	ns	0.6752
	DS patient 1 vs Control 3	***	0.0007	*	0.03	****	<0.0001
	DS patient 5 vs Control 1	*	0.0337	***	0.0005	*	0.0126
	DS patient 5 vs Control 2	ns	0.3484	ns	0.1416	**	0.0032
	DS patient 5 vs Control 3	ns	0.2786	ns	0.7587	ns	0.6038
	DS patient 1 vs DS patient 5	ns	0.0596	ns	0.2041	*	0.0152
	Control 1 vs Control 2	ns	0.9748	ns	0.7063	ns	0.8217
	Control 1 vs Control 3	***	0.0005	****	<0.0001	****	<0.0001
	Control 2 vs Control 3	*	0.0406	*	0.0297	****	<0.0001
DIV75	DS patient 1 vs Control 1	ns	0.9375	ns	0.7673	ns	0.8607
	DS patient 1 vs Control 2	ns	0.8081	ns	0.7622	ns	0.8022
	DS patient 1 vs Control 3	**	0.0026	*	0.0472	**	0.0027
	DS patient 5 vs Control 1	ns	0.9938	**	0.0014	*	0.029
	DS patient 5 vs Control 2	ns	0.9804	ns	0.3517	ns	0.0553
	DS patient 5 vs Control 3	ns	0.5002	ns	0.9981	ns	0.4907
	DS patient 1 vs DS patient 5	ns	>0.9999	ns	0.0795	ns	0.2161
	Control 1 vs Control 2	ns	0.4244	ns	0.0946	ns	0.9971
	Control 1 vs Control 3	***	0.0007	***	0.0007	***	0.0002
	Control 2 vs Control 3	ns	0.1829	ns	0.2182	**	0.0011
DIV82	DS patient 1 vs Control 1	ns	0.9999	ns	0.7081	ns	0.9983
	DS patient 1 vs Control 2	**	0.0047	**	0.0075	ns	0.467
	DS patient 1 vs Control 3	ns	0.8507	ns	0.1712	ns	0.0986
	DS patient 5 vs Control 1	ns	0.5892	*	0.0495	ns	0.3587
	DS patient 5 vs Control 2	ns	0.0699	ns	0.528	ns	0.9846
	DS patient 5 vs Control 3	ns	>0.9999	ns	0.9914	ns	0.8647
	DS patient 1 vs DS patient 5	ns	0.7037	ns	0.4121	ns	0.5651
	Control 1 vs Control 2	**	0.0013	***	0.0004	ns	0.3394
	Control 1 vs Control 3	ns	0.7719	*	0.014	*	0.0397
	Control 2 vs Control 3	ns	0.1638	ns	0.7947	ns	0.9991
DIV89	DS patient 1 vs Control 1	ns	0.9663	ns	0.1105	ns	0.9994
	DS patient 1 vs Control 2	**	0.0053	***	0.0001	ns	0.2831
	DS patient 1 vs Control 3	ns	0.9912	ns	0.9731	ns	0.844
	DS patient 5 vs Control 1	ns	0.9361	ns	0.086	ns	0.1447
	DS patient 5 vs Control 2	ns	0.0749	ns	0.0947	ns	0.9731

	DS patient 5 vs Control 3	ns	0.9997	ns	>0.9999	ns	0.8753
	DS patient 1 vs DS patient 5	ns	0.9994	ns	0.9762	ns	0.2713
	Control 1 vs Control 2	***	0.0003	****	<0.0001	ns	0.2084
	Control 1 vs Control 3	ns	0.8476	ns	0.074	ns	0.6968
	Control 2 vs Control 3	ns	0.1069	ns	0.0603	ns	0.699

MFR=mean firing rate, nbursts= number of bursts, Sig = significance, Adj. p = adjusted p-value. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant

Supplementary Table S8: Statistics of mean burst duration, burst per min in bursts detecting electrodes and number of active electrodes in GABAergic enriched cultures.

Tukey's multiple comparisons test		Supplementary Figure 2C		Supplementary Figure 2D		Supplementary Figure 2E	
		Mean burst duration		**Burst per min in bursts detecting electrodes**		**number of active electrodes**	
		Sig	Adj. p	Sig	Adj. p	Sig	Adj. p
DIV19	**-**	-	-	-	-	-	-
DIV26	DS patient 1 vs Control 1	ns	0.59	ns	0.9203	ns	>0.9999
	DS patient 1 vs Control 2	ns	0.3419	ns	0.487	ns	0.621
	DS patient 1 vs Control 3	ns	>0.9999	***	0.0006	ns	0.5318
	DS patient 5 vs Control 1	ns	0.7013	ns	0.9993	ns	0.9891
	DS patient 5 vs Control 2	ns	0.5728	ns	0.8496	ns	0.8496
	DS patient 5 vs Control 3	ns	0.988	***	0.0005	ns	0.999
	DS patient 1 vs DS patient 5	ns	0.9905	ns	0.9896	ns	0.9927
	Control 1 vs Control 2	ns	0.971	ns	0.5629	ns	0.6299
	Control 1 vs Control 3	ns	0.1192	***	0.0005	ns	0.4143
	Control 2 vs Control 3	*	0.026	***	0.0004	****	<0.0001
DIV33	DS patient 1 vs Control 1	ns	>0.9999	ns	0.9991	ns	0.9472
	DS patient 1 vs Control 2	ns	0.9984	ns	0.9617	ns	0.3458
	DS patient 1 vs Control 3	ns	>0.9999	ns	>0.9999	****	<0.0001
	DS patient 5 vs Control 1	ns	0.6306	ns	0.9997	ns	0.9966
	DS patient 5 vs Control 2	ns	0.9831	ns	0.4869	*	0.0151
	DS patient 5 vs Control 3	ns	0.5163	ns	0.7156	***	0.0003
	DS patient 1 vs DS patient 5	ns	0.9098	ns	0.9271	ns	0.7338
	Control 1 vs Control 2	ns	0.9961	ns	0.9757	ns	0.1209
	Control 1 vs Control 3	ns	0.9995	ns	0.9971	***	0.0004
	Control 2 vs Control 3	ns	0.999	ns	0.8933	****	<0.0001
DIV40	DS patient 1 vs Control 1	ns	0.8797	ns	0.9799	****	<0.0001
	DS patient 1 vs Control 2	ns	0.7671	ns	0.5344	*	0.0139
	DS patient 1 vs Control 3	ns	0.8088	ns	0.8448	****	<0.0001

	DS patient 5 vs Control 1	ns	0.7571	ns	0.4215	ns	0.15
	DS patient 5 vs Control 2	ns	0.3685	*	0.0213	ns	0.9975
	DS patient 5 vs Control 3	ns	0.501	ns	0.1177	ns	0.1279
	DS patient 1 vs DS patient 5	ns	0.9937	ns	0.8692	**	0.0016
	Control 1 vs Control 2	ns	0.9556	ns	0.7315	ns	0.4318
	Control 1 vs Control 3	ns	0.9916	ns	0.9879	ns	0.9999
	Control 2 vs Control 3	ns	0.999	ns	0.8081	ns	0.3754
DIV47	DS patient 1 vs Control 1	ns	0.1862	ns	0.6366	***	0.0003
	DS patient 1 vs Control 2	*	0.0131	ns	0.5518	**	0.0081
	DS patient 1 vs Control 3	ns	0.0769	*	0.017	ns	0.9884
	DS patient 5 vs Control 1	ns	0.9868	ns	0.8814	****	<0.0001
	DS patient 5 vs Control 2	ns	0.2673	ns	0.7897	****	<0.0001
	DS patient 5 vs Control 3	ns	0.8169	ns	0.9047	ns	0.088
	DS patient 1 vs DS patient 5	ns	0.4227	ns	0.1689	ns	0.2773
	Control 1 vs Control 2	ns	0.3944	ns	>0.9999	ns	0.7162
	Control 1 vs Control 3	ns	0.9623	ns	0.3124	**	0.0012
	Control 2 vs Control 3	ns	0.8459	ns	0.1097	*	0.0271
DIV54	DS patient 1 vs Control 1	ns	0.8863	ns	0.1273	**	0.0017
	DS patient 1 vs Control 2	ns	0.2849	ns	0.838	ns	0.4443
	DS patient 1 vs Control 3	**	0.0094	**	0.0046	ns	0.9985
	DS patient 5 vs Control 1	ns	0.9997	ns	0.8713	****	<0.0001
	DS patient 5 vs Control 2	ns	0.7824	ns	0.9998	**	0.0077
	DS patient 5 vs Control 3	ns	0.1016	ns	0.2343	ns	0.2505
	DS patient 1 vs DS patient 5	ns	0.9809	ns	0.8797	ns	0.0838
	Control 1 vs Control 2	ns	0.5085	ns	0.4108	ns	0.514
	Control 1 vs Control 3	**	0.0015	ns	0.2738	**	0.0021
	Control 2 vs Control 3	*	0.0338	*	0.0109	ns	0.3737
DIV61	DS patient 1 vs Control 1	ns	0.9992	ns	0.8392	**	0.0015
	DS patient 1 vs Control 2	ns	0.0836	ns	0.926	ns	0.6669
	DS patient 1 vs Control 3	*	0.0364	ns	0.6993	*	0.0448
	DS patient 5 vs Control 1	*	0.0411	ns	0.8838	****	<0.0001
	DS patient 5 vs Control 2	ns	0.9761	ns	0.9435	**	0.0085
	DS patient 5 vs Control 3	ns	>0.9999	ns	0.7452	ns	0.9996
	DS patient 1 vs DS patient 5	ns	0.0921	ns	>0.9999	*	0.0202
	Control 1 vs Control 2	**	0.0092	ns	0.9976	ns	0.2808
	Control 1 vs Control 3	**	0.0049	ns	0.9745	****	<0.0001
	Control 2 vs Control 3	ns	0.8541	ns	0.9254	*	0.0134
DIV68	DS patient 1 vs Control 1	ns	0.9979	ns	0.1192	**	0.0011
	DS patient 1 vs Control 2	*	0.0488	ns	0.9345	ns	0.9335
	DS patient 1 vs Control 3	ns	0.5108	ns	0.1634	*	0.0424
	DS patient 5 vs Control 1	**	0.0061	ns	0.1419	****	<0.0001
	DS patient 5 vs Control 2	ns	0.9504	ns	0.9877	**	0.0065
	DS patient 5 vs Control 3	ns	0.8347	ns	0.2156	ns	0.9061

	DS patient 1 vs DS patient 5	*	0.0277	ns	0.996	**	0.0021
	Control 1 vs Control 2	*	0.0108	ns	0.5577	ns	0.0536
	Control 1 vs Control 3	ns	0.5596	ns	0.9986	****	<0.0001
	Control 2 vs Control 3	ns	0.949	ns	0.5598	*	0.0354
DIV75	DS patient 1 vs Control 1	ns	0.492	*	0.0174	***	0.0005
	DS patient 1 vs Control 2	ns	0.1995	ns	0.3168	ns	0.9898
	DS patient 1 vs Control 3	ns	>0.9999	ns	0.3498	ns	0.234
	DS patient 5 vs Control 1	ns	0.9896	ns	0.9757	****	<0.0001
	DS patient 5 vs Control 2	ns	0.7714	ns	0.9925	ns	0.265
	DS patient 5 vs Control 3	ns	0.9263	ns	>0.9999	ns	0.9809
	DS patient 1 vs DS patient 5	ns	0.9298	ns	0.3573	*	0.011
	Control 1 vs Control 2	**	0.0035	ns	0.4952	**	0.0012
	Control 1 vs Control 3	ns	0.6772	ns	0.9958	****	<0.0001
	Control 2 vs Control 3	ns	0.6104	ns	0.9794	ns	0.6719
DIV82	DS patient 1 vs Control 1	ns	0.9998	**	0.003	***	0.0002
	DS patient 1 vs Control 2	***	0.0009	ns	0.1781	*	0.0195
	DS patient 1 vs Control 3	ns	0.9911	ns	0.7486	ns	0.7772
	DS patient 5 vs Control 1	ns	0.9161	ns	0.553	****	<0.0001
	DS patient 5 vs Control 2	ns	0.3741	ns	0.9576	ns	0.6795
	DS patient 5 vs Control 3	ns	0.8863	ns	>0.9999	ns	0.961
	DS patient 1 vs DS patient 5	ns	0.9314	ns	0.3597	ns	0.2606
	Control 1 vs Control 2	***	0.001	ns	0.992	****	<0.0001
	Control 1 vs Control 3	ns	0.9982	ns	0.8668	****	<0.0001
	Control 2 vs Control 3	*	0.0244	ns	0.9806	ns	0.3516
DIV89	DS patient 1 vs Control 1	ns	0.9903	ns	0.1277	*	0.0148
	DS patient 1 vs Control 2	ns	0.0589	*	0.0119	***	0.0009
	DS patient 1 vs Control 3	ns	0.964	ns	0.2762	ns	0.983
	DS patient 5 vs Control 1	ns	0.9946	ns	0.994	**	0.0041
	DS patient 5 vs Control 2	ns	0.6043	ns	0.3918	ns	0.1562
	DS patient 5 vs Control 3	ns	0.7369	ns	0.9786	ns	0.9899
	DS patient 1 vs DS patient 5	ns	0.9425	ns	0.7389	ns	0.8544
	Control 1 vs Control 2	ns	0.1279	ns	0.2877	****	<0.0001
	Control 1 vs Control 3	ns	0.8307	ns	0.9975	**	0.0081
	Control 2 vs Control 3	*	0.0452	ns	0.7024	*	0.0227

Sig = significance, Adj. p = adjusted p-value. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant

Supplementary Table S9: Statistics of MFR for active electrodes, nbursts and percentages of spikes in bursts in heterogenous cortical cultures.

Tukey's multiple comparisons test	Figure 4A		Figure 4B		Figure 4C	
	MFR for active electrodes		**nburst**		**Percentages of spikes in burts**	
	Sig	Adj. p	Sig	Adj. p	Sig	Adj. p

DIV41	DS patient 1 vs DS patient 5	****	<0.0001	***	0.0001	ns	0.9997
	DS patient 1 vs Control 4	ns	0.9197	ns	0.6543	ns	0.8995
	DS patient 1 vs Control 2	ns	0.1063	ns	0.9804	ns	0.9238
	DS patient 5 vs Control 4	**	0.0037	ns	0.1542	ns	0.9006
	DS patient 5 vs Control 2	****	<0.0001	**	0.0023	ns	0.9713
	Control 4 vs Control 2	ns	0.2316	ns	0.8479	ns	0.641
DIV48	DS patient 1 vs DS patient 5	ns	0.0858	ns	0.2286	ns	0.1179
	DS patient 1 vs Control 4	**	0.0074	***	0.001	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 4	***	0.0010	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	ns	0.8865	*	0.0277	ns	0.9897
DIV55	DS patient 1 vs DS patient 5	ns	0.5984	ns	0.9984	ns	0.3652
	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	***	0.0007	ns	0.4665	***	0.0003
DIV62	DS patient 1 vs DS patient 5	ns	0.9955	ns	0.0524	ns	0.2631
	DS patient 1 vs Control 4	****	<0.0001	***	0.0002	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	*	0.0337	****	<0.0001
	DS patient 5 vs Control 4	****	<0.0001	****	<0.0001	***	0.0001
	DS patient 5 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	ns	0.4007	ns	0.0602	ns	0.1319
DIV69	DS patient 1 vs DS patient 5	*	0.0375	ns	0.8508	*	0.0214
	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 4	***	0.0001	****	<0.0001	ns	0.0829
	DS patient 5 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	ns	0.9975	ns	0.0662	**	0.0054
DIV76	DS patient 1 vs DS patient 5	*	0.0194	ns	0.4935	***	0.0001
	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 4	*	0.0237	****	<0.0001	ns	0.7007
	DS patient 5 vs Control 2	ns	0.6647	***	0.0004	****	<0.0001
	Control 4 vs Control 2	ns	0.1163	**	0.007	*	0.0125
DIV83	DS patient 1 vs DS patient 5	*	0.0173	ns	0.5818	***	0.0006
	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 1 vs Control 2	**	0.0048	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 4	***	0.0005	****	<0.0001	*	0.0251
	DS patient 5 vs Control 2	ns	0.4373	***	0.0006	*	0.0303
	Control 4 vs Control 2	ns	0.3279	**	0.001	ns	0.9979
DIV90	DS patient 1 vs DS patient 5	*	0.0111	*	0.0303	****	<0.0001

	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	****	<0.0001
	DS patient 1 vs Control 2	***	0.0008	****	<0.0001	***	0.0003
	DS patient 5 vs Control 4	***	0.0002	****	<0.0001	ns	0.3595
	DS patient 5 vs Control 2	ns	0.9996	**	0.0075	ns	>0.9999
	Control 4 vs Control 2	****	<0.0001	**	0.0075	ns	0.5786

MFR=mean firing rate, nbursts= number of bursts, Sig = significance, Adj. p = adjusted p-value. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant

Supplementary Table S10: Statistics of mean burst duration, burst per min in bursts detecting electrodes and Inter-burst interval in heterogenous cortical cultures.

Tukey's multiple comparisons test		Supplementary Figure 4B		Supplementary Figure 4C		Supplementary Figure 4D	
		Mean burst duration		Burst per min in bursts detecting electrodes		Inter-burst interval	
		Sig	Adj. p	Sig	Adj. p	Sig	Adj. p
DIV41	DS patient 1 vs DS patient 5	ns	0.6148	***	0.0001	ns	0.8184
	DS patient 1 vs Control 4	ns	0.4502	ns	0.6507	ns	>0.9999
	DS patient 1 vs Control 2	ns	0.8174	**	0.0097	ns	0.8993
	DS patient 5 vs Control 4	ns	0.4873	*	0.019	ns	0.9331
	DS patient 5 vs Control 2	ns	0.9974	ns	0.1987	ns	0.164
	Control 4 vs Control 2	ns	0.4793	ns	0.06	ns	0.9481
DIV48	DS patient 1 vs DS patient 5	ns	0.72	ns	0.5295	ns	0.6162
	DS patient 1 vs Control 4	**	0.0071	ns	0.1769	ns	0.0634
	DS patient 1 vs Control 2	ns	0.1251	****	<0.0001	*	0.0201
	DS patient 5 vs Control 4	*	0.0265	***	0.0005	*	0.0419
	DS patient 5 vs Control 2	ns	0.5893	****	<0.0001	**	0.0014
	Control 4 vs Control 2	ns	0.0849	***	0.0007	ns	0.2643
DIV55	DS patient 1 vs DS patient 5	ns	0.8448	ns	0.7292	ns	0.1439
	DS patient 1 vs Control 4	****	<0.0001	**	0.0085	**	0.0015
	DS patient 1 vs Control 2	****	<0.0001	**	0.0022	***	0.0008
	DS patient 5 vs Control 4	****	<0.0001	ns	0.0915	ns	0.0554
	DS patient 5 vs Control 2	****	<0.0001	ns	0.1524	*	0.0151
	Control 4 vs Control 2	ns	0.2647	ns	0.7454	ns	>0.9999
DIV62	DS patient 1 vs DS patient 5	ns	0.0906	ns	0.4625	ns	0.5723
	DS patient 1 vs Control 4	****	<0.0001	ns	0.1727	*	0.0236
	DS patient 1 vs Control 2	****	<0.0001	ns	0.8882	ns	0.0524
	DS patient 5 vs Control 4	***	0.0002	**	0.0047	*	0.0178
	DS patient 5 vs Control 2	**	0.0025	ns	0.3472	ns	0.0522
	Control 4 vs Control 2	*	0.0156	*	0.0191	ns	0.5119
DIV69	DS patient 1 vs DS patient 5	*	0.0285	ns	0.1973	ns	0.6365

	DS patient 1 vs Control 4	****	<0.0001	****	<0.0001	***	0.0002
	DS patient 1 vs Control 2	****	<0.0001	ns	0.0666	*	0.0147
	DS patient 5 vs Control 4	ns	0.0609	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 2	ns	0.6958	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	ns	0.1818	**	0.0015	****	<0.0001
DIV76	DS patient 1 vs DS patient 5	*	0.0367	ns	0.9179	ns	0.8273
	DS patient 1 vs Control 4	***	0.0003	****	<0.0001	ns	0.097
	DS patient 1 vs Control 2	*	0.033	***	0.0003	**	0.0013
	DS patient 5 vs Control 4	ns	0.9975	****	<0.0001	*	0.037
	DS patient 5 vs Control 2	ns	0.4253	****	<0.0001	**	0.0026
	Control 4 vs Control 2	ns	0.0645	**	0.0014	ns	0.9919
DIV83	DS patient 1 vs DS patient 5	ns	0.0507	ns	0.4149	ns	0.6614
	DS patient 1 vs Control 4	****	<0.0001	**	0.0042	****	<0.0001
	DS patient 1 vs Control 2	ns	0.0503	ns	0.559	***	0.0004
	DS patient 5 vs Control 4	ns	0.8265	****	<0.0001	ns	0.0655
	DS patient 5 vs Control 2	ns	0.9622	****	<0.0001	ns	0.1378
	Control 4 vs Control 2	ns	0.3561	**	0.0032	**	0.002
DIV90	DS patient 1 vs DS patient 5	**	0.0092	ns	0.1377	ns	0.9831
	DS patient 1 vs Control 4	****	<0.0001	**	0.0052	**	0.0032
	DS patient 1 vs Control 2	ns	0.8941	ns	0.3435	ns	0.2213
	DS patient 5 vs Control 4	ns	0.0817	****	<0.0001	****	<0.0001
	DS patient 5 vs Control 2	ns	0.0643	***	0.0009	*	0.0485
	Control 4 vs Control 2	****	<0.0001	ns	0.2243	ns	0.1184

Sig = significance, Adj. p = adjusted p-value. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant

Supplementary Table S11: Statistics of Mean spike frequency in bursts and number of active electrodes in heterogenous cortical cultures.

Tukey's multiple comparisons test		Supplementary Figure 4I		Supplementary Figure 4J	
		Mean spike frequency in bursts		**number of active electrodes**	
		Sig	Adj. p	Sig	Adj. p
DIV41	DS patient 1 vs DS patient 5	ns	0.9644	****	<0.0001
	DS patient 1 vs Control 4	ns	0.9982	ns	0.3736
	DS patient 1 vs Control 2	ns	0.813	ns	0.761
	DS patient 5 vs Control 4	ns	0.9736	ns	0.1011
	DS patient 5 vs Control 2	ns	0.9847	ns	0.1056
	Control 4 vs Control 2	ns	0.9038	ns	0.9841
DIV48	DS patient 1 vs DS patient 5	ns	0.8019	ns	0.1145
	DS patient 1 vs Control 4	ns	0.9771	*	0.0393

	DS patient 1 vs Control 2	****	<0.0001	ns	0.9791
	DS patient 5 vs Control 4	ns	0.629	***	0.0004
	DS patient 5 vs Control 2	****	<0.0001	ns	0.4523
	Control 4 vs Control 2	****	<0.0001	*	0.0404
DIV55	DS patient 1 vs DS patient 5	ns	0.4663	*	0.0233
	DS patient 1 vs Control 4	ns	0.3046	ns	0.5805
	DS patient 1 vs Control 2	****	<0.0001	ns	0.112
	DS patient 5 vs Control 4	ns	0.9794	**	0.0073
	DS patient 5 vs Control 2	****	<0.0001	****	<0.0001
	Control 4 vs Control 2	****	<0.0001	ns	0.9998
DIV62	DS patient 1 vs DS patient 5	ns	0.726	ns	0.467
	DS patient 1 vs Control 4	**	0.0092	**	0.0089
	DS patient 1 vs Control 2	****	<0.0001	ns	0.1163
	DS patient 5 vs Control 4	ns	0.5417	***	0.0001
	DS patient 5 vs Control 2	****	<0.0001	**	0.0012
	Control 4 vs Control 2	****	<0.0001	ns	0.4235
DIV69	DS patient 1 vs DS patient 5	ns	0.0846	ns	0.7547
	DS patient 1 vs Control 4	***	0.0001	***	0.0009
	DS patient 1 vs Control 2	****	<0.0001	**	0.0055
	DS patient 5 vs Control 4	ns	0.8337	****	<0.0001
	DS patient 5 vs Control 2	****	<0.0001	***	0.0004
	Control 4 vs Control 2	****	<0.0001	ns	0.8432
DIV76	DS patient 1 vs DS patient 5	**	0.0012	ns	0.9996
	DS patient 1 vs Control 4	ns	0.1371	***	0.0002
	DS patient 1 vs Control 2	****	<0.0001	ns	0.2364
	DS patient 5 vs Control 4	ns	0.8855	***	0.0003
	DS patient 5 vs Control 2	***	0.0006	ns	0.247
	Control 4 vs Control 2	ns	0.634	ns	0.1341
DIV83	DS patient 1 vs DS patient 5	*	0.0206	ns	0.9968
	DS patient 1 vs Control 4	***	0.0005	****	<0.0001
	DS patient 1 vs Control 2	****	<0.0001	ns	0.1777
	DS patient 5 vs Control 4	ns	0.8072	****	<0.0001
	DS patient 5 vs Control 2	***	0.0004	ns	0.1634
	Control 4 vs Control 2	**	0.003	ns	0.0926
DIV90	DS patient 1 vs DS patient 5	ns	0.134	*	0.0291
	DS patient 1 vs Control 4	**	0.002	****	<0.0001
	DS patient 1 vs Control 2	**	0.006	*	0.0271
	DS patient 5 vs Control 4	ns	0.7176	****	<0.0001
	DS patient 5 vs Control 2	ns	0.8247	ns	0.7857
	Control 4 vs Control 2	ns	0.9976	**	0.0033

Sig = significance, Adj. p = adjusted p-value. Statistical significance is denoted as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant